



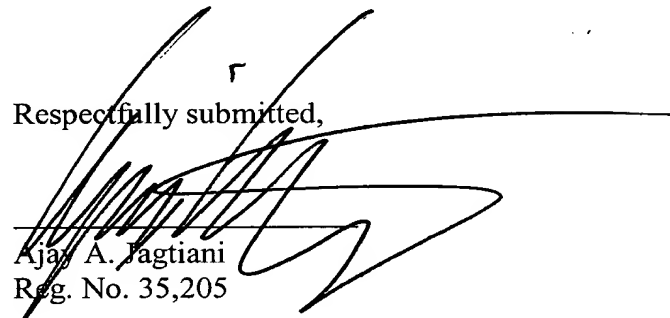
Serial Number: 09/424,181

REMARKS

The attached paper and computer readable copies of the Sequence Listing are submitted in compliance with C.F.R. 1.821-1.825. The contents of the paper and computer readable copies of the Sequence Listing are the same. No new matter is added.

Early and favorable consideration on the merits is respectfully requested.

Respectfully submitted,



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Attachments:

Sequence Listing (paper and computer readable copies)

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September 7, 2001



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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In re Application of:)Box:	SEQUENCE
ROGELJ ET AL.)	
SERIAL NUMBER: 09/424,181)	
FILED: NOVEMBER 10, 1999)Art Unit:	1653
FOR: INHIBITION OF CELL SURFACE PROTEIN)	
DISULFIDE ISOMERASE)Examiner:	LUKTON, D
)DOCKET No:	UNME-0054-1

Director of the U.S. Patent and Trademark Office
Washington, D.C. 20231

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Sir:

Below are the amendments for the above-identified application shown in redlined format:

IN THE SPECIFICATION:

Please amend the Specification, without prejudice or disclaimer, as indicated below:

Please insert the attached Sequence Listing, pages 1, at the end of the Application.

Please delete the paragraph at page 1, line 19 to page 2, line 9 and substitute therefor the following paragraph:

PDI (protein disulfide isomerase) is a constitutive intracellular protein that is also found to be expressed on the surface of many mammalian cell types, including immune system cells, hepatocytes, and platelets. Like other members of the thyredoxin superfamily of proteins, PDI is a multifunctional redox-sensitive protein

that catalyzes oxidation-reduction reactions via a vicinal dithiol-dependent disulfide-sulfhydryl interchange between its internal vicinal dithiol (Cys-Gly-His-Cys SEQ ID NO. 1) active sites and the disulfide bonds of its substrates to promote their reconfiguration. PDI recognizes the side chains of cysteine residues in its substrates, and it is its two vicinal dithiol groups, one or two on each of two identical PDI subunits, that are critical for its enzymatic isomerase function, in particular its broad specificity for correcting the configuration of a large spectrum of proteins as needed. For example, PDI is present in the endoplasmic reticulum of most cells, where it is believed to mediate co- and post-translational modifications of nascent proteins with incorrect sulfide bonds; it is also present in certain protein complexes such as triglyceride transfer protein complex (MTP) wherein it maintains the complex in a catalytically-active state and inhibits complex aggregation. Membrane PDI catalyzes the cleavage of disulfide bonds during the earliest stages of endocytosis, and activates diphtheria toxin by catalyzing cleavage of this disulfide-linked dimer. PDI also catalyzes the isomerization of thrombospondin (TSP) disulfide bonds, thereby profoundly modulating TSP-ligand binding activity. Both TSP and PDI are released by activated platelets; PDI is also released by degranulated neutrophils (J. Cell Physiol. 144:280, 1990).

Please delete the paragraph at page 6, lines 16 to 25 and substitute therefor the following paragraph:

According to the invention, cell-surface PDI (csPDI) isomerase activity is effectively inhibited by thiol blocking agents (inhibitors) which covalently or non-covalently cross-link two or more free vicinal sulfhydryl groups of one or more PDI active site peptide sequences to form complexes stable in the cell environment. The -SH groups of the cysteine residues in the sequence Cys-Gly-His-Cys (SEQ ID NO. 1) are exemplary. The inhibitors are preferably highly selective for PDI vicinal sulfhydryls and have sufficient affinity for these groups to compete successfully with

the ligand to be denied access to these sites and prevent PDI-mediated isomerization of its disulfide bonds and its consequent reconfiguration for undesired biological activity. The sequence of PDI is known (Nature 317:6034; 267, 1985) Herein, "csPDI" and "PDI" are used interchangeably unless otherwise noted.

Please delete the paragraph at page 12, lines 4 to 14 and substitute therefor the following paragraph:

Most of the inhibitors identified by the inventors to date, including cadmium, and trivalent arsenical and antimonial compounds work by blocking the vicinal cysteines in PDI active sites; however, some inhibitors may work by blocking PDI activity by a mechanism that is different from the thiol-mediated blockade of the Cys-Gly-His-Cys (SEQ ID NO. 1) active sites. The inhibitors are generally not cell-specific (unlike, for example, fMLP for which CHO and lymphocytes are receptor negative), and are selected as the application requires as described herein. Cell-membrane impermeable inhibitors are typically selected for applications requiring minimization of toxicity as are the dithiol and dithiol-specific inhibitors, as these tend to be efficacious at lower relative concentrations. Monothiol and/or cell-membrane permeable inhibitors are, however, useful in the practice of the invention and may prove equal or superior to dithiol inhibitors in applications where a slight increase in cell toxicity is not a critical factor.